OCT 19 2001

Oey, Jan

From: Jill Schultz [jill.schultz@Covance.Com]
Sent: Friday, October 19, 2001 10:01 AM
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Subject: Nicotine Glucuronide validation data

[redacted] nicotine.xls [redacted] cotinine.xls [redacted] pmgluc-1.xls

Attached are the tabulated validation data for the glucuronide assay and summary sheets. These data are not 100% QC checked at this stage, hence they may be subject to minor change. Please review and contact me with any questions or your authorization to proceed with sample analysis.

Mark had the following comments to add regarding these data:

- Water blank samples containing internal standard (technically the zero calibration standard which is not used in the regression) for nicotine typically contains an amount equivalent to between 20-40% of the LLOQ, which was also apparent during the aglycone validation. Normal practice would advocate raising the LLOQ for this analyte to a level whereby this interference is always less than 20%. However, on a within batch basis, such interference has been very consistent, reflected by the fact that acceptable precision and accuracy has been achieved at all levels down to the LLOQ of 1 ng/mL. It is suspected that background levels for nicotine derive, at least in part, from the solid phase extraction sorbent. I would recommend that the LLOQ remain at 1 ng/mL, this phenomenon being discussed within the validation reports.

- Dilution QCs were diluted with control urine instead of water, as stated in the protocol, prior to analysis. This does not affect the data, however an amendment will be required to document this protocol deviation.

- Intra and inter-day data would suggest that acceptance limits of 20% (rather than 15%) for precision and accuracy of low QC samples during sample analysis may be more realistic for the glucuronides...for consideration by PM.

- Stability data demonstrate that the glucuronides are stable stored at room temperature for 24 h and subjected to 3 additional freeze/thaw

cycles. However, the apparent percent aglycone liberated by nicotine glucuronide 2 ng/mL QC samples taken through the SPE method (referenced against deconjugated 2 ng/mL QCs), using peak area ratios, was in the region of 33%. I believe this to be an artefact due to quantifying area ratios below the LLOQ, compounded by the issue raised in the first paragraph above. If degradation was occurring, noticeable aglycone concentrations would be expected from glucuronide QCs prepared at high concentrations (not apparent).

- Stock solution stability will be assessed when solutions are prepared for the sample analysis phase, to save on expensive standards.

- Please note that Mark will be on vacation 10/23 through 11/11. Phil Turpin will serve as his backup while he is away.

Regards,

Jill
